

Characterization of Biochemically Atypical *Vibrio cholerae* Strains and Designation of a New Pathogenic Species, *Vibrio mimicus*

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Biochemically atypical strains classified as *Vibrio cholerae* were characterized by biochemical reactions, serology, antibiotic susceptibility testing, and deoxyribonucleic acid relatedness. Strains with the following atypical reactions were shown to be *V. cholerae*: mannose negative, mannitol negative, lysine decarboxylase negative, no growth in the presence of 5% NaCl, salicin and cellobiose positive. Sucrose-negative strains were shown to constitute a new species, *Vibrio mimicus*, whose type strain is 1721-77 (ATCC 33653). In addition to its negative sucrose reaction, *V. mimicus* was differentiated from *V. cholerae* by its negative Voges-Proskauer, corn oil, and Jordan tartrate reactions and by its sensitivity to polymyxin. *V. mimicus* was isolated from shellfish and water, as well as from human diarrheal stools and ear infections. Most strains were typable with antisera against *V. cholerae*. Strains from three serogroups produced either a heat-labile or a heat-stable enterotoxin.

Vibrio cholerae has long been known as the etiological agent of cholera. It was first thought that only the classical biotype of *V. cholerae* serotype O:1 caused cholera, but the eltor biotype was subsequently shown to cause the disease. Serotypes other than O:1 were called non-agglutinating vibrios or noncholera vibrios. Their role in disease was not recognized, and despite their biochemical similarity to O:1 strains they were often not considered as *V. cholerae*.

As early as 1935, Garner and Venkatraman demonstrated that O:1 and non-O:1 *V. cholerae* strains had identical H antigens (9). Many investigators have shown that non-O:1 strains cause sporadic cases and even occasional epidemics of cholera. This observation was confirmed and extended by Sakazaki and Shimada (15). Sakazaki et al. studied the biochemical reactions of a large number of non-O:1 strains, concluding that they were *V. cholerae* (14). Their phenotypic observations were confirmed by Citarella and Colwell (5), who showed that O:1 and non-O:1 strains of *V. cholerae* were inseparable on the basis of deoxyribonucleic acid

(DNA) relatedness.

Hugh and Sakazaki published a minimal set of biochemical characteristics with which to define *V. cholerae* (12). These minimal diagnostic characteristics were subsequently reaffirmed by the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Vibrios (13).

The original purpose of this study was to define the biochemical parameters of *V. cholerae* by determining DNA relatedness of biochemically atypical strains to a typical strain of *V. cholerae*. During the investigation, we found that sucrose-negative strains were not *V. cholerae*, but a new species, for which the name *Vibrio mimicus* sp. nov. is proposed. The characteristics of *V. mimicus* are presented, and strain 1721-77 (ATCC 33653) is designated as the type strain.

MATERIALS AND METHODS

Bacterial strains. Fifty-one strains of *V. mimicus*, as well as other biochemically atypical strains of *V. cholerae* used in DNA relatedness studies, are listed in Table 1. The biochemical reactions of these strains

TABLE 1. *Bacterial strains*

Strain	Source ^a	Origin	Smith serotype ^b
<i>V. cholerae</i>			
9060-79 (typical)	ATCC 14035, type strain	India	O:1
9061-79 (typical)	ATCC 14033	Egypt	O:1
1196-78 (typical)	Sewage	Brazil	O:1
5062 (mannose ⁻)		H. L. Smith	15
7165 (mannose ⁻)		H. L. Smith	201
5011 (no growth in 5% NaCl)		H. L. Smith	333
1528-79 (mannitol ⁻)	Oysters	Texas	O:1
1727-79 (mannitol ⁻)	Oysters	Louisiana	O:1
1742-79 (mannitol ⁻)	Oysters	Texas	Untypable
1936-79 (lysine ⁻)	Human diarrheal stool	California	O:1
1954-79 (mannitol ⁻)	Oysters	Texas	O:1
1955-79 (mannitol ⁻)	Oysters	Texas	O:1
1956-79 (mannitol ⁻)	Oysters	Texas	O:1
2088-78 (salicin ⁺ , cellobiose ⁺)	Human stool	Bangladesh	42
<i>V. mimicus</i>			
6306	Human stool	H. L. Smith	107
6358	Water	H. L. Smith	160
6661	Human stool	H. L. Smith	113
7629	Water mite	H. L. Smith	42
548-77	Human stool	Guam	329
1721-77	Human, ear infection	North Carolina	106
3560-77	Human, diarrheal stool (consumed raw oysters)	Louisiana	15
517-78	Human, diarrheal stool	Mexico	113
1638-78	Human, diarrheal stool	Louisiana	74
1726-78	Human stool	Louisiana	Untypable
2074-78	Human stool	Bangladesh	Rough
2079-78	Human stool	Bangladesh	42
2080-78	Human stool	Bangladesh	115
2082-78	Human stool	Bangladesh	106
2575-78	Human, otitis	New Zealand	106
3145-78	Human, diarrheal stool	Orient	106
732-79	Human ear	Massachusetts	325
739-79	Water	Maryland	42
740-79	Human, diarrheal stool	Bangladesh	23
741-79	Human, diarrheal stool	Bangladesh	113
743-79	Human, diarrheal stool	Philippines	42
744-79	Water	Bangladesh	56
745-79	Human, patient contact	Bangladesh	23
746-79	Unknown	Bangladesh	Untypable
748-79	Human, diarrheal stool	Bangladesh	106
2266-79	Human stool	Kansas	113
2520-79	Human diarrheal stool	Ohio	15
11-80	Human, ear	Canada	42
269-80	Water	Louisiana	43
781-80	Human stool (food poisoning from shellfish)	Louisiana	113
782-80	Human stool (food poisoning from shellfish)	Louisiana	113
783-80	Human stool (food poisoning from shellfish)	Louisiana	113
951-80	Water	Louisiana	Rough
954-80	Water	Louisiana	Rough
960-80	Oyster	Florida	309
968-80	Prawn	New Zealand	24
979-80	Human wound	New Zealand	23

TABLE 1—Continued

Strain	Source ^a	Origin	Smith serotype ^b
1138-80	Human, stool	Bangladesh	23
1139-80	Human, stool	Bangladesh	23
1141-80	Oyster	Maryland	106
1142-80	Oyster	New York	106
1143-80	Oyster	Louisiana	113
1144-80	Oyster	Virginia	42
1145-80	Oyster	Louisiana	12
1146-80	Oyster	Maryland	106
1248-80	Water	Massachusetts	Untypable
1673-80	Oyster	M. Presnell, FDA, ^c Alabama	107
1674-80	Water	M. Presnell, FDA, Mississippi	68
1755-80 (6306)	Human, diarrheal stool	Bangladesh	107
1756-80 (6358)	Water	Bangladesh	160
1758-80 (7629)	Water nuts	Bangladesh	42
<i>V. alginolyticus</i> 9065-79	ATCC 17749, type strain		
<i>V. fischeri</i>	ATCC 7744, type strain		
<i>V. metschnikovii</i> 9529-78	NCTC 11170	A. Furniss (strain VL 2484)	
<i>V. parahaemolyticus</i> 9062-79	ATCC 17802, type strain		
<i>V. parahaemolyticus</i> 1754-80	CDC laboratory strain		
<i>V. vulnificus</i> 1675-80	CDC laboratory strain		
<i>V. vulnificus</i> 1779-80	CDC laboratory strain		
<i>Aeromonas hydrophila</i> 747-79	CDC laboratory strain		
<i>Escherichia coli</i> K-12	CDC DNA hybridization reference strain		

^a Diarrhea or other disease is noted when present. No notation of illness for human isolates indicates that data were not available.

^b Untypable, No reaction in Smith antisera.

^c FDA, Food and Drug Administration.

were compared to the reactions of approximately 300 *V. cholerae* strains submitted to the Enteric Section at the Centers for Disease Control, Atlanta, Ga., between 1975 and 1980.

Media biochemical and serological tests. Dehydrated media from commercial sources were used whenever possible. Except for the tyrosine clearing test (11), media preparation and test conditions were those of Edwards and Ewing (8), as updated by Hickman and Farmer (10). The string test was done as described by Smith (17). Sensitivity to the vibriostatic pteridine compound 0/129 (16) was done on disks prepared as follows: sterile 6-mm filter paper disks were soaked in a 0.1% solution of 2,4-diamino-6,7-diisopropylpteridine (Calbiochem, La Jolla, Calif.) in acetone and then dried overnight under a laminar flow hood. Sensitivity to polymyxin (50-U disks) was done by the disk method of Bauer et al. (1). Occasional strains of *V. cholerae* and most strains of many other *Vibrio* species grow poorly or not at all in the absence of salt. Media as normally used for fermentation of carbohydrates and for most other tests contained 0.5% NaCl. A final concentration of 1% NaCl was added to those test media that do not contain salt: oxidase, indole, methyl red, Voges-Proskauer, nitrate reduction, gelatin, esculin, lysine, arginine, and ornithine.

Salt tolerance was determined by the ability of a strain to grow in nutrient broth containing 0, 1, 6, 8, 10, and 12% NaCl. O antigens were determined with antisera prepared by H. L. Smith, Jr., by using the Smith typing system (18, 19).

Enterotoxin assays. Cultures were tested for elaboration of heat-labile enterotoxin by either the Y-1 adrenal cell assay (7) or the enzyme-linked immunosorbent assay (20); for elaboration of heat-stable enterotoxin, cultures were tested by the infant mouse assay (6).

Antibiotic susceptibility testing. Antibigrams were done by the disk method of Bauer et al (1), with the concentrations of antibiotics previously published (11). The results of antibigrams obtained from 23 *V. mimicus* strains were compared with antibigrams from 85 typical *V. cholerae* strains selected from both human and environmental sources. All zone sizes were measured in millimeters.

DNA hybridization. The procedures used for DNA hybridization have been described (2-4). With few exceptions, DNAs from strains of any given species are 70% or more related at conditions optimal for DNA reassociation (in this case, 60°C incubations), and 60% or more related at supraoptimal reassociation criteria (in this case, 75°C incubations).

TABLE 2. Biochemical reactions of *V. mimicus* and *V. cholerae*

Biochemical or test	Cumulative % positive at day: ^a					
	<i>V. mimicus</i>			<i>V. cholerae</i>		
	1	2	7	1	2	7
Indole		88			81	
Methyl red		15			24	
Voges-Proskauer		0			65	
Citrate (Simmons)	93	95	95	91	96	98
H ₂ S on triple sugar iron agar	0	0	0	0	0	0
Urea	0	0	7	0	0	4
Phenylalanine deaminase	0			0.3		
Lysine decarboxylase	85	95	98	98	98	99
Arginine dihydrolase	0	0	0	0	0	0
Ornithine decarboxylase	88	95	98	98	98	99
Motility	98	100	100	95	97	98
Gelatin (22°C)	24	61	90	16	43	76
KCN, growth in	0	0	0	2	11	18
Malonate	0	0	10	0.3	2	44
D-Glucose acid	100	100	100	99.7	100	100
D-Glucose gas	0	0	0	0.3	0.3	0.3
Acid from:						
Adonitol	0	0	0	0	0	0
L-Arabinose	0	2	2	0	0	0
D-Arabitol	0	0	0	0	0	0
Cellobiose	2	2	2	8	8	10
Dulcitol	0	0	2	0.3	0.3	3.0
Erythritol	0	0	0	0	0	0
Galactose	70	80	97	80	90	92
Glycerol	2	10	39	5	32	52
<i>i</i> -Inositol	0	0	0	0	0	0
Lactose	0	24	56	2	9	74
Maltose	98	98	100	99.7	100	100
D-Mannitol	95	98	100	91	98	98
D-Mannose	90	98	100	87	88	88
Melibiose	0	0	2	0.3	0.7	0.7
α -CH ₃ -D-glucoside	0	0	0	0	0	0
Raffinose	0	0	0	0	0	0
L-Rhamnose	0	0	0	0	0	0
Salicin	2	2	2	4	5	6
D-Sorbitol	0	0	0	0	0	0
Sucrose	0	0	0	99.7	100	100
Trehalose	100	100	100	88	96	99
D-Xylose	0	0	0	0	0	0
Esculin	0	0	0	0	0	1
Mucate	0	0	0	0	0	0
Jordan tartrate	4	5	5	50	63	64
Acetate	34	75	88	70	95	98
Lipase (corn oil)	9	10	12	92	95	95
Deoxyribonuclease (25°C)	44	78	93	73	92	99
NO ₃ ⁻ → NO ₂ ⁻	92			99		
Oxidase	95			100		
ONPG ^b	85	85	90	93	94	96
H ₂ S on peptone iron agar	0	0	0	0	0	0
Tyrosine clearing	5	27	43	3	21	28
Pectate	0	0	0	0	0	0
Citrate (Christensen)	90	93	95	91	98	98
String test	100			99.7		
Polymyxin, 50 U, sensitivity	87			22		
0/129 test, sensitivity	98			99		

TABLE 2—Continued

Biochemical or test	Cumulative % positive at day: ^a					
	<i>V. mimicus</i>			<i>V. cholerae</i>		
	1	2	7	1	2	7
Tests to which NaCl is added:						
Indole		93			81	
Methyl red		93			99	
Voges-Proskauer		0			65	
Lysine decarboxylase	100	100	100	98	99	99
Arginine dihydrolase	0	0	0	0	0	0
Ornithine decarboxylase	93	93	100	98	99	100
Gelatin (22°C)	32	65	97	11	36	74
Esculin	0	0	3	1	1	2
NO ₃ ⁻ → NO ₂ ⁻	100			99		
Oxidase	100			99		
Nutrient broth, growth 0% NaCl	92	95	95	97	99	100
Nutrient broth, growth 1% NaCl	100	100	100	99	100	100
Nutrient broth, growth 6% NaCl	27	51	54	21	52	60
Nutrient broth, growth 8% NaCl	0	0	8	0	1	2
Nutrient broth, growth 10% NaCl	0	0	0	0	0	0
Nutrient broth, growth, 12% NaCl	0	0	0	0.4	0.4	0.4

^a Blank space indicates not determined. An occasional strain grew poorly or not at all in media that lacked salt. These tests were considered negative.

^b ONPG, *o*-Nitrophenyl- β -D-galactopyranoside.

RESULTS

Biochemical tests. Biochemical reactions of *V. cholerae* and *V. mimicus* are given in Table 2. Since occasional strains of both species grow poorly or not at all in some media that lack salt, tests done both in the presence and absence of added NaCl are presented. All tested strains of *V. mimicus* were sucrose negative, whereas all *V. cholerae* strains were sucrose positive. Other tests of value in separating *V. mimicus* and *V. cholerae* were Voges-Proskauer (0 and 65% positive, respectively), corn oil (10 and 95% positive, respectively, within 48 h), Jordan tartrate (5 and 63% positive, respectively, within 48 h), and polymyxin sensitivity (87 and 22% sensitive, respectively).

O antigens and enterotoxin production. O antigen typing and enterotoxin assays were done on 51 *V. mimicus* strains (Table 3). Forty-five strains were typable in Smith's *V. cholerae* O antisera. The most common serogroups were 106, 113, 42, and 23. Three strains could not be typed with Smith's antisera, and three strains were rough. Enterotoxin production, as judged by positive response in the Y-1 adrenal cell assay or enzyme-linked immunosorbent assay for heat-labile enterotoxin or in the infant mouse assay for heat-stable enterotoxin, was detected in eight strains. The enterotoxigenic strains were restricted to serotypes 106, 113, and 23. No strain was positive for both heat-labile and heat-stable enterotoxin.

Antibiotic susceptibility. The antibiotic susceptibility patterns of *V. mimicus* and *V. cholerae* are given in Table 4. *V. mimicus* strains were susceptible to all antibiotics tested except for sulfadiazine, although single strains were resistant to colistin, nalidixic acid, and kanamycin, and three strains were resistant to penicillin. The susceptibility pattern of *V. mimicus* was very similar to that of *V. cholerae*, except for colistin, to which most *V. cholerae* strains showed resistance.

DNA relatedness. *V. cholerae* 9061-79 (ATCC 14033), the reference strain for biotype eltor, was chosen as the reference strain for DNA relatedness studies. Strain 9061-79 was chosen in preference to the type strain of *V. cholerae* 9060-79 (ATCC 14035), which is a "classical" biotype, because most strains isolated in the United States are biotype eltor. DNA relatedness of labeled *V. cholerae* 9061-79 DNA to unlabeled DNAs from *V. cholerae* and *V. mimicus* strains is shown in Table 5. Relatedness between strains 9061-79 and 9060-79 is 94%, with less than 2% divergence in related sequences. The lowest relatedness, only 64%, was with another biochemically typical *V. cholerae* strain, 1196-78, from Brazil; however, divergence was less than 1% and relatedness remained at 64% in reactions done at 75°C. The biochemically atypical strains were all highly related to strain 9061-79 in 60°C reactions (73 to 92% range, 87% average), and relatedness remained high in 75°C

reactions (66 to 84% range, 79% average). The divergence in related sequences was between 0.6 and 2.6%. Therefore, strains that are mannose negative, mannitol negative, lysine decarboxylase negative, unable to grow in 5% NaCl, or salicin and cellobiose positive are *V. cholerae*.

The five *V. mimicus* strains shown in Table 5

TABLE 3. *O* antigens and enterotoxin production in *V. mimicus*^a

O antigen	No. of strains	Enterotoxin production	
		Heat labile	Heat stable
106	8		1
113	7	1	1
42	7		
23	5	4	1
107	3		
15	2		
160	2		
12	1		
24	1		
43	1		
56	1		
68	1		
74	1		
113	1		
115	1		
309	1		
325	1		
329	1		
Untypable	3		
Rough	3		

^a One strain of O antigen group 42 and the single strains of O antigen groups 43 and 106 caused a cytopathogenic effect in the Y-1 adrenal cell assay for heat-labile enterotoxin. These results were considered negative for enterotoxin production.

were originally tested as representing sucrose-negative strains of *V. cholerae* belonging to Heiberg group 5 (sucrose negative, arabinose negative, mannose positive). They were not related to *V. cholerae* at the species level (species level is defined in our laboratory as 70% or more relatedness at optimal, in this case 60°C, reassociation temperature, 5% or less divergence, and 50% or more relatedness at supraoptimal, in this case 75°C, reassociation temperature).

On the basis of these data, 52 sucrose-negative strains were tested by DNA relatedness to determine whether they formed one or more than one relatedness group and whether any sucrose-negative strains belonged to *V. cholerae*. The results clearly indicate that *V. mimicus* encompasses all of the sucrose-negative strains (Table 6). *V. mimicus* is more closely related to *V. cholerae* than to the other *Vibrio* species tested, or to *Aeromonas hydrophila*.

Taxonomic description. The name *Vibrio mimicus* sp. nov. is proposed for the sucrose-negative, cholera-like strains: min. ic' us, M.L. noun *mimicus* to mimic, because of its similarity to *V. cholerae*. The type strain of *V. mimicus* is 1721-77 (ATCC 33653). *V. mimicus* is a gram-negative, oxidase-positive, vibrio-shaped bacterium that is motile by means of a single polar flagellum (Fig. 1). It is positive in the string test, is sensitive to polymyxin and to the vibriocidal agent 0/129, and grows in the absence of NaCl and in 1% NaCl; approximately 50% of the strains grow in 6% NaCl. Negative reactions for sucrose fermentation, Voges-Proskauer, and lipase (10% positive) are the most useful diagnostic tests in separating *V. mimicus* from *V. cholerae*. The overall biochemical reactions for *V. mimicus* are shown in Table 2, and its antibiotic

TABLE 4. Antibiotic susceptibility of *V. mimicus* and *V. cholerae*

Antibiotic	Mean zone diam (mm)	<i>V. mimicus</i> (23 strains)		Mean zone diam (μm)	<i>V. cholerae</i> (85 strains)	
		SD ^a	% Resist-ant ^b		SD	% Resist-ant
Tetracycline	24	2	0	24	2	0
Colistin	11	1	4	7	2	84
Nalidixic acid	26	4	4	27	3	1
Sulfadiazine	7	3	87	11	7	69
Gentamicin	21	2	0	20	2	0
Streptomycin	16	2	0	15	2	5
Kanamycin	19	2	4	19	3	4
Chloramphenicol	28	3	0	27	3	1
Penicillin	15	3	13	14	2	8
Ampicillin	18	2	0	17	2	0
Carbenicillin	24	2	0	23	2	0
Cephalothin	23	2	0	22	2	1

^a SD, Standard deviation.

^b For the purpose of this study, "intermediate" zone sizes were considered to be susceptible.

TABLE 5. DNA relatedness of atypical *V. cholerae* and of *V. mimicus* strains to *V. cholerae* 9061-79

Source of unlabeled DNA ^a	RBR ^b at 60°C	%D ^c	RBR at 75°C
<i>Vibrio cholerae</i> 9061-79	100	0	100
<i>V. cholerae</i> 9060-79	94	1.7	
<i>V. cholerae</i> 1196-78	64	0.6	64
<i>V. cholerae</i> 5062 (man ⁻)	73	1.1	66
<i>V. cholerae</i> 7165 (man ⁻)	85	0.7	76
<i>V. cholerae</i> 5011 (NaCl ⁻)	80	0.6	78
<i>V. cholerae</i> 1528-79 (mtl ⁻)	87	1.9	80
<i>V. cholerae</i> 1727-79 (mtl ⁻)	87	1.1	79
<i>V. cholerae</i> 1742-79 (mtl ⁻)	90	2.2	80
<i>V. cholerae</i> 1954-79 (mtl ⁻)	92	1.7	84
<i>V. cholerae</i> 1955-79 (mtl ⁻)	88	1.8	82
<i>V. cholerae</i> 1956-79 (mtl ⁻)	90	1.4	84
<i>V. cholerae</i> 1936-79 (lys ⁻)	91	2.6	83
<i>V. cholerae</i> 2088-78 (sal ⁺ , cel ⁺)	92	2.4	81
<i>V. mimicus</i> 6306	54	7.7	24
<i>V. mimicus</i> 6358	48	8.4	26
<i>V. mimicus</i> 7629	35	6.8	23
<i>V. mimicus</i> 6661	27	8.3	
<i>V. mimicus</i> 5634	24	4.9	18
<i>Escherichia coli</i> K-12	3	16.7	

^a man, Mannose; mtl, mannitol; lys, lysine decarboxylase.

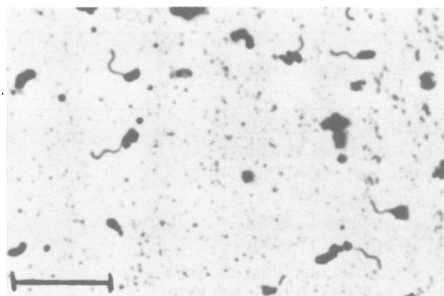
^b RBR, Relative binding ratio = percent heterologous DNA reaction/percent homologous DNA reaction \times 100.

^c %D, Percentage of divergence; calculated on the assumption that each 1°C decrease in the thermal stability of a DNA duplex is caused by 1% of unpaired bases within that duplex. All reactions were done at least twice. Control reactions without added unlabeled DNA showed RBR values of 4 to 6%. The actual binding in homologous *V. cholerae* 9061-79 reactions was between 60 and 70%.

susceptibility profile is shown in Table 4. Most strains are typable in antisera produced against *V. cholerae* (Table 1). *V. mimicus* has been isolated frequently from water and shellfish, from human diarrheal stools, and ear infections (Table 1). Some strains produce either heat-labile or heat-stable enterotoxin (Table 3).

DISCUSSION

The results of this study dictate a redefinition of the biochemical parameters of *V. cholerae* and the establishment of a new species, *V. mimicus*. We have shown by DNA relatedness that aberrant or unusual reactions for salt tolerance, lysine decarboxylase, mannitol, mannose, salicin, and cellobiose are permissible within the

FIG. 1. Photomicrograph of *V. mimicus*. Bar, 10 μ m.

confines of *V. cholerae*. These data are a logical extension of the data proving that serotypes other than O:1 are *V. cholerae* (4). There are few, if any, reactions that can be used a priori to include or to exclude a strain from a given species. Experience with *Enterobacteriaceae* has shown to our satisfaction that a phenotypic definition of species, in the absence of DNA relatedness data, is incorrect as often as it is correct. *V. mimicus* is yet another example of the necessity of DNA relatedness in defining the biochemical parameters of species. Without DNA relatedness data, how could one include mannose-negative, arabinose-negative, sucrose-positive strains (Heiberg group 2) in *V. cholerae* and exclude sucrose-negative, arabinose-negative, mannose-positive strains (Heiberg group 5) from *V. cholerae*?

V. mimicus has been isolated with increasing frequency from American waters and shellfish during the past 2 years. It has also been isolated from Bangladesh, Mexico, New Zealand, Guam, Canada, and the Orient. This increase in isolates is undoubtedly due to the increased awareness of and culturing for *V. cholerae* in the United States. There has not been an epidemiological study of *V. mimicus* isolates; however, the data on source of isolation leave little doubt of its implication in acute diarrhea linked to the consumption of shellfish (Table 1). It is also quite likely that *V. mimicus*, like other *Vibrio* species, is an etiological agent of ear infections.

Most *V. mimicus* strains are typable by using antisera produced against *V. cholerae*. The 45 typable strains in our collection belong to 18 O groups, three strains were not typable, and three were rough (Table 3). Thirty-four strains (76% of total typable) belong to just seven O groups, and 27 of these (60% of total typable) belong to just four O groups.

Five strains produced heat-labile and three produced heat-stable enterotoxin. The toxigenic

TABLE 6. DNA relatedness in *V. mimicus*^a

Source of unlabeled DNA	Labeled DNA from <i>V. mimicus</i> 1721-77		
	RBR ^b at 60°C	%D ^c	RBR at 75°C
<i>Vibrio mimicus</i> 1721-77	100	0	100
51 <i>V. mimicus</i> strains	91(82-99) ^d	1.7(0.5-3.1)	91(78-97)
<i>V. cholerae</i> 9060-79	67	9.2	40
<i>V. cholerae</i> 9061-79	67	8.8	39
<i>V. vulnificus</i> 1779-80	40	12.2	18
<i>V. vulnificus</i> 1765-80	34	16.3	11
<i>V. parahaemolyticus</i> 1754-80	36	14.6	14
<i>V. parahaemolyticus</i> 9062-79	32	14.6	7
<i>V. alginolyticus</i> 9065-79	26	15.8	6
<i>V. metschnikovii</i> 9529-78	28	15.7	6
<i>V. fischeri</i> 9064-79	21	14.9	7
<i>Aeromonas hydrophila</i> 747-79	15		6
<i>Escherichia coli</i> K-12	9		2

^a All reactions were done at least twice. Control reactions without added unlabeled DNA showed RBR values of 1 to 4%. The actual binding in homologous *V. mimicus* 1721-77 reactions were 70 and 89%.

^{b,c} See Table 5, footnotes *b* and *c*, for definitions of RBR and *D*.

^d Range of values obtained.

strains were restricted to serogroups 113, 106, and 23. All five isolates of serogroup 23 were toxigenic. The heat-labile-enterotoxin-positive strains were all from human stools, one heat-stable enterotoxin strain was from a wound culture, and the other was from a stool culture. *V. mimicus* certainly appears to be pathogenic for humans.

Nine *V. mimicus* O groups were isolated from stools. Five of six extraintestinal human isolates and six of nine shellfish isolates possessed the same O antigens found in stool isolates. In contrast, only two of eleven strains isolated from water had O antigens found in human strains.

Additional strains must be studied to better determine the incidence of *V. mimicus* in the environment and in humans. The role of enterotoxin as a virulence factor in the disease also needs further investigation, as does the seeming correlation between O antigens and disease.

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